FIGURE 1A

Nucleotide sequence of NS2/3 (810-1206) st (SEQ ID. No. 1)

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accttgtcac catactataa agtgctcctc gctaggctca tatggtggtt acagtattta
atcaccagag togaggogca cttgcaagtg tggatccccc ctctcaatgt tcggggaggc
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ttcgtgcgtg cgcaggggct cattcgtgcg tgtatgttgg tgcggaaggc tgcggggggt
cattatgtcc aaatggcctt catgaagcta gctgcgctga caggtacgta cgtttatgac
catctcactc cattgcagga ttgggcccac gcgggcctac gagaccttgc agtggcggta
qaqcccqtca tcttctctqa catgqaqqtc aaqatcatca cctqqqqqqc qqacaccqcq
qcatgcgggg acatcatttc aggtctgccc gtctccgctc gaaggggaag ggagatactc
ctgggaccgg ccgataattt tgaagggcag gggtggcgac tccttgcgcc catcacggcc
tactcccaac agacacgggg cctacttggt tgcatcatca ccagcctcac aggccgggac
aagaaccagg tcgaggggga ggttcaagtg gtctccaccg ctacacaatc tttcctggcg
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qqccccaaaq qcccaatcac ccaqatqtac actaatqtqq accaqqacct cqtcqqctqq
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ttggtcacga gacatgccga cgtcattccg gtgcgccggc ggggcgacag tagggggagc
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ccttcggggc acgttgtggg catcttccgg gctgctgtgt gcacccgggg ggttgcaaaa
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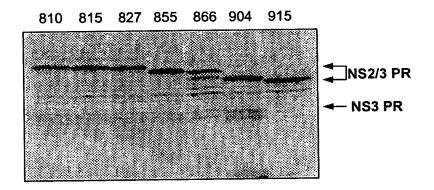
FIGURE 1B

AMINO ACID SEQUENCE OF NS2/3 (810-1206)-ST (SEQ ID NO. 2)

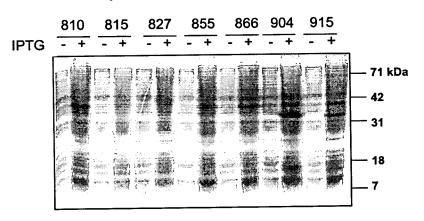
MDREMAASCG GAVFIGLALL TLSPYYKVLL ARLIWWLQYL ITRVEAHLQV WIPPLNVRGG RDAIILLTCA
VHPELIFDIT KLLLAIFGPL MVLQAGITKV PYFVRAQGLI RACMLVRKAA GGHYVQMAFM KLAALTGTYV
YDHLTPLQDW AHAGLRDLAV AVEPVIFSDM EVKIITWGAD TAACGDIISG LPVSARRGRE ILLGPADNFE
GQGWRLLAPI TAYSQQTRGL LGCIITSLTG RDKNQVEGEV QVVSTATQSF LATCVNGVCW TVFHGAGSKT
LAGPKGPITQ MYTNVDQDLV GWQAPPGARS MTPCTCGSSD LYLVTRHADV IPVRRGDSR GSLLSPRPVS
YLKGSSGGPL LCPSGHAVGI FRAAVCTRGV AKAVDFIPVE SMETTMRTSS AWRHPOFGG

FIGURE 2

A. in vitro translated N-terminal deletion mutants



B. E. coli expression - SDS-PAGE



C. E. coli expression - Immunoblot with anti-NS3

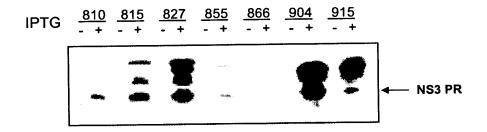
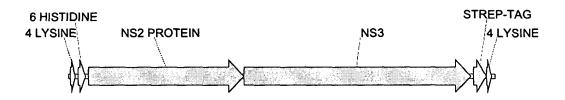
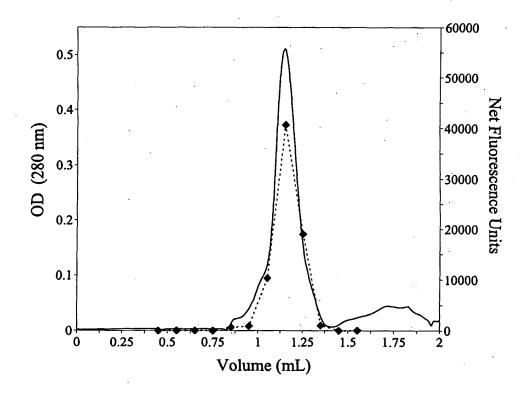


FIGURE 3



4k 6H NS2/3(904-1206)-ST-4K

FIGURE 4



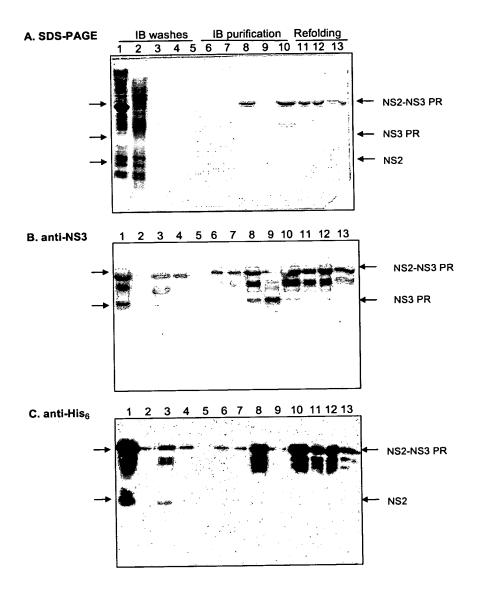


FIGURE 6

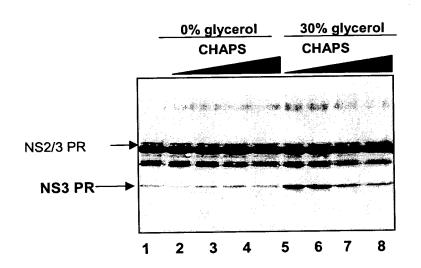


FIGURE 7

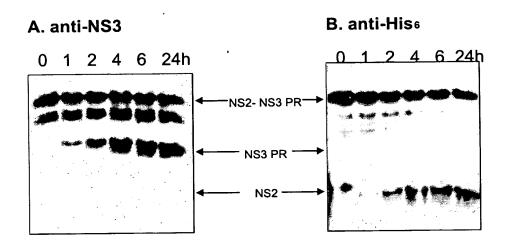


FIGURE 8

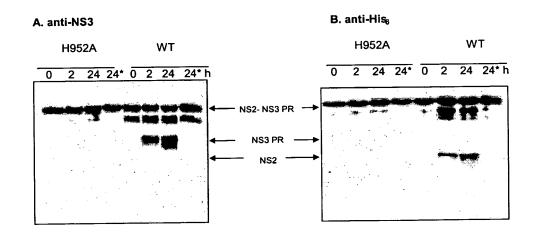


FIGURE 9A

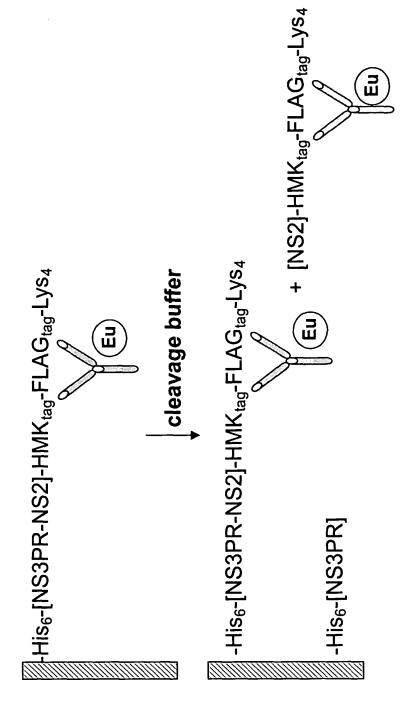
NUCLEOTIDE SEQUENCE OF 4K-6H-NS2/3 (904-1206) ST-4K (SEQ ID NO. 3)

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gcggggggtc attatgtcca aatggccttc atgaagctag ctgcgctgac aggtacgtac
gtttatgacc atctcactcc attgcaggat tgggcccacg cgggcctacg agaccttgca
gtggcggtag agcccgtcat cttctctgac atggaggtca agatcatcac ctgggggggg
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ggccgggaca agaaccaggt cgagggggag gttcaagtgg tctccaccgc tacacaatct
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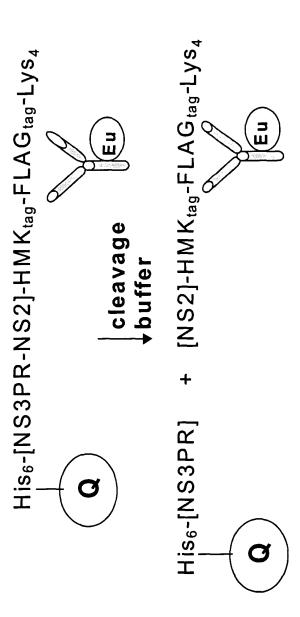
FIGURE 9B

AMINO ACID SEQUENCE OF 4K-6H-NS2/3 (904-1206) ST-4K (SEQ ID NO. 4)

MKKKKLEHHH	HHHTSAGITK	VPYFVRAQGL	IRACMLVRKA	AGGHYVQMAF	MKLAALTGTY	60
VYDHLTPLQD	WAHAGLRDLA	VAVEPVIFSD	MEVKIITWGA	DTAACGDIIS	GLPVSARRGR	120
EILLGPADNF	EGQGWRLLAP	ITAYSQQTRG	LLGCIITSLT	GRDKNQVEGE	VQVVSTATQS	180
FLATCVNGVC	WTVFHGAGSK	TLAGPKGPIT	QMYTNVDQDL	VGWQAPPGAR	SMTPCTCGSS	240
DLYLVTRHAD	VIPVRRRGDS	RGSLLSPRPV	SYLKGSSGGP	LLCPSGHAVG	IFRAAVCTRG	300
WAKAWDETDW	ESMETTMRTS	SAWRHPOFGG	KKKK			337



8/12



9/12

His₆-[NS3PR-NS2]-HMK_{tag}-FLAG_{tag}-Lys₄

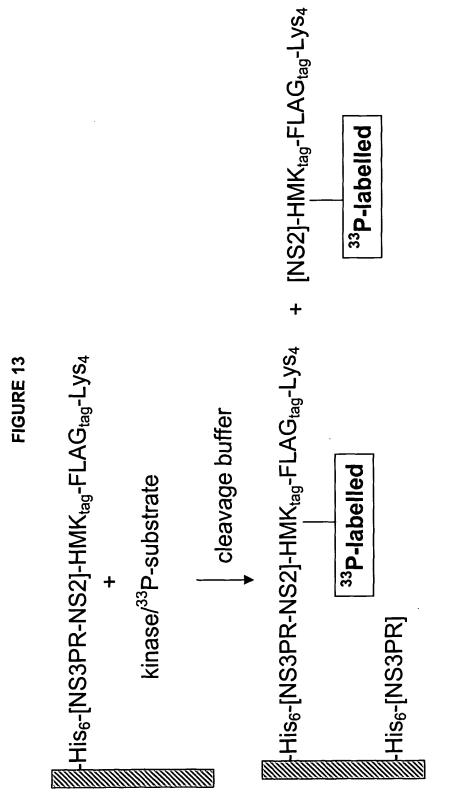
fluorescent probe (potent NS3 protease inhibitor)

cleavage buffer

His₆-[NS3PR] His₆-[NS3P_R-NS2]-HMK_{tag}-FLAG_{tag}-Lys₄ +



+ [NS2]-HMK_{tag}-FLAG_{tag}-Lys₄



11/12

50 mM Hepes, pH 7.5, 30% glycerol, 0.5% n-dodecyl-β-D-maltoside, 1 mM TCEP Strep_{tag}-[NS3PR-NS2]-His₆-Lys₄ ر د د

Strep_{tag}-[NS3PR-NS2]-His₆-Lys₄ + Strep_{tag}-[NS3PR] + [NS2]-His₆-Lys₄



Streptavidin coated plate



Eu⁺³-labeled anti-NS2 (signal decrease assay)

1 h washes

TRF detection